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FOREWORD

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INTRODUCTION

This is the Final Report for this project. It provides new updated information since the last report, which was submitted in June 2004, and provides an overall summary of progress, and remaining steps required to meet the study objectives. Although those objectives were not completed during the funding period, they will be completed using other funding sources. The objective of this case-control study is to determine whether oxidative damage is a risk factor for prostate cancer, and whether this mechanism mediates the association between dietary fat and prostate cancer risk. Specifically, cases and controls will be compared with respect to malondialdehyde (MDA) in serum as a measure of oxidative stress, and deoxyguanosine malondialdehyde (dG-MDA) in peripheral lymphocytes and prostate tumor samples as a measure of oxidative DNA damage. In addition to these measures, dietary intake of fats and specific fatty acids, and of antioxidants will be considered as potential effect modifiers or confounding factors, as will serum antioxidant levels. Enrollment of cases and controls, collection of questionnaire data, and collection of serum, plasma and lymphocytes has been completed. Remaining tasks are:

1. Analyze serum and DNA for measures of oxidative stress (serum) or oxidative DNA damage (DNA), and for levels of antioxidants (serum).
2. Conduct statistical analyses of risk associated with oxidative damage, and whether it mediates effects of dietary factors such as fats or antioxidants.
3. Publish manuscripts detailing the study findings.

As has been described in previous reports, the study was transferred from Georgetown University to Johns Hopkins in 2001. There was a long period of inactivity while the study protocol and consent form were being negotiated between the Department of Defense, the Principal Investigator, and Johns Hopkins School of Medicine. The issues were successfully resolved and the study was re-activated in Jan 2003. Enrollment of additional cases and controls continues to the present at Johns Hopkins.

The population enrolled in this case-control study also formed the basis for successful funding of a project in the NCI-funded Johns Hopkins Prostate Specialized Program of Research Excellence (SPORE) award (SPORE Grant #2 P50 CA58236-10, "Project 5: DNA polymorphisms in genes affecting levels of oxidative stress in prostate cells: population studies of association with prostate cancer risk"). That study will build on the existing case-control dataset and analyze a series of single nucleotide polymorphisms (SNPs) associated with generation of or response to oxidative stress, to determine whether any are risk factors for prostate cancer, or modify the risk associated with oxidative stress.

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An additional small pilot project has been initiated with Dr. Prakash Rao at the University of South Alabama. This study measured leptin in the serum of a subset of prostate cancer cases and controls from the parent study. Leptin is a potential risk factor for prostate cancer that may also modify the association of diet with risk. These samples have been analyzed.

BODY

Study Progress

This section will describe the following:

- (a) original study objectives
- (b) delays in re-activating the study at Johns Hopkins
- (c) progress since the study was re-activated
- (d) plans for concluding the study.

Study Objectives.

Task 1. To complete enrollment of prostate cancer cases scheduled to undergo prostatectomy, and benign urologic surgery controls, from urology clinics at Johns Hopkins University School of Medicine (JHU), Georgetown University (GU), the Veteran's Administration Medical Center (VA) in Washington, DC, and the Washington Hospital Center (WHC). This includes collection of serum, plasma, lymphocytes, epidemiologic and dietary intake data, and (from cases) paraffin-embedded tumor tissue.

Task 2. To measure the following biomarkers:

- Serum malondialdehyde
- Malondialdehyde deoxyguanosine adducts in peripheral blood lymphocytes
- Malondialdehyde deoxyguanosine adducts in prostate tumor tissue
- Complete serum fatty acid profile
- Serum antioxidants including alpha-tocopherol (vitamin E) and carotenoids
- Serum androgens and related hormones or metabolites, including testosterone, dihydrotestosterone, 3 α androstenediol glucuronide, and SHBG

Task 3. To conduct a case-control study with the above data to compare the levels of lipid peroxidation biomarkers in cases with controls, to determine the following:

- Whether lipid peroxidation levels modify the association of dietary fat with prostate cancer risk.
- Whether lipid peroxidation biomarkers in serum (MDA) or lymphocytes (dGMDA adducts) provide a good estimate of the extent of oxidative DNA damage in prostate tumors (dGMDA adducts in tumor).
- Whether lipid peroxidation levels are a function of dietary fat and androgens.

Change in Institutions. In April 2001 the Principal Investigator, Dr. Bruce Trock, moved from Georgetown University (GU) to Johns Hopkins University (JHU). The plan at that time was to

continue to enroll patients from the original enrollment sites at GU, the Veteran's Administration Medical Center (VA) in Washington, DC, and Washington Hospital Center (WHC), and to also begin enrolling patients from JHU.

Delays in re-activating the study. This was described in detail in the Annual Report dated June 2004 and will only be summarized here. An initial delay resulted from suspension of all research activity and acceptance of new IRB applications at JHU in July 2001 due to the death of a subject in a research study completely unrelated to Dr. Trock's research. Subsequent, more prolonged delays resulted from problems concerning the original consent form. Dr. Trock thought that the awarding of the grant and funds signified approval to begin and so began enrolling patients. In fact, the original informed consent form had never been approved by USAMRAA, and the study was not authorized to enroll patients. Dr. Trock was informed of this in September 2001 and suspended study activities as requested. Following this a long series of communications took place, during which Dr. Trock provided evidence that no research subjects had been harmed in any way, and the entire grant proposal, study protocol, and consent form were re-reviewed. Ultimately, everything was resolved, the consent form was revised, and the study was authorized by USAMRAA to begin in January 2003. This Human Subjects Review process was assigned HSRRB Log Number A-09282, and all communications during that process are referenced to that number.

Progress since re-activation of the study. Following reactivation, the emphasis was on completing recruitment of cases and controls. Control recruitment was slower than we had anticipated because, despite the very large patient volumes seen by the Brady Urological Institute, a large fraction of patients being seen for reasons other than prostate cancer (i.e. potential controls) had current or prior history of other cancers (including non-urologic cancers), and were thus, ineligible. The requirement that controls have documented PSA < 2.5 and a normal DRE also reduced the number of eligible patients. We currently enroll two different types of controls. Patients coming for routine urology care unrelated to malignancy and with no history of cancer form the first group ("true controls"). However, despite the fact that these patients will have had a recent (within 2 years) PSA < 2.5 ng/ml and a normal DRE, some of these patients will harbor undetected prostate cancer (Thompson 2004). For this reason we also include among the controls men who have had a recent negative prostate biopsy, and are not considered to be at "high risk" of being a false negative (i.e. PSA ≤ 8 ng/ml, no evidence of prostate intraepithelial neoplasia (PIN) or atypia on biopsy, and normal digital rectal exam. These men have about a 20% chance of having prostate cancer missed by the biopsy (false negative). We follow these men for one year to identify any who have a subsequent biopsy that identifies cancer or high grade PIN (HGPIN); such men would be switched from the control population to the case population.

The following table summarizes our recruitment of cases and controls to date, showing results from the different clinical sites:

	Cases (n=411)	Controls (n=360)
JHU	248	biopsy negative: 30, true control: 245
GU, VA, WHC	163	biopsy negative: 73, true control: 12
blood available	361	biopsy negative: 97, true control: 247
questionnaire(s) available	376	biopsy negative: 89, true control: 223
white	277	biopsy negative: 68, true control: 219
black	106	biopsy negative: 21, true control: 15
asian	5	biopsy negative: 1, true control: 7
Hispanic	6	biopsy negative: 0, true control: 1
other	10	biopsy negative: 3, true control: 5
Unknown	7	Biopsy negative: 10, true control: 10

Since our last report (June 2004) we have enrolled an additional 91 cases and 110 controls. Thus, we have exceeded the targeted enrollment of 240-300 cases and 240-300 controls. 16% of subjects are African American, with a higher proportion among cases (25%) than controls (10%). This reflects our early experience with the primary source of patients coming from the Washington VA Hospital, which has a large proportion of African American patients. Because recruitment at the VA took place early in the study, we primarily identified cases, since our entry point to the system was through the prostate biopsy clinic. In contrast, most recruitment of controls has taken place at Johns Hopkins, where the majority of patients are white.

Serum, plasma and lymphocytes are available from 91% of enrolled patients and epidemiology/dietary questionnaire data are available for 89%. We are continuing to contact patients who have not yet returned their questionnaires to obtain additional data. Included among the cases are data from 81 patients with premalignant lesions, i.e. subjects with HGPIN and atypia. Such patients are known to have a very high risk of subsequent diagnosis of prostate cancer. They may be of interest because, if oxidative damage truly is a risk factor for prostate cancer, these may exhibit levels intermediate between those of cancer cases and controls.

We have submitted dietary data questionnaires (Block food frequency instrument) from approximately 70% of patients for nutrient analysis. These are submitted in batches; the final batch will be submitted this month. These analyses are performed by Berkley Nutrition Services, the company that also produces the Block food frequency instrument. We have also submitted an initial batch of serum and lymphocyte DNA samples from 50 cases and 50 controls to our

collaborator, Dr. A. Venket Rao at University of Toronto, for analysis of serum MDA, dG-MDA DNA adducts, and serum antioxidants. These analyses were delayed several months because Dr. Rao was out of the lab for an extended period due to travel, then an injury, and his lab tech who performs these analyses was on maternity leave. He recently returned and we plan to submit the remainder of the samples in batches, approximately twice per month.

A major consideration in the evaluation of any case-control study is the comparability of the case and control populations. In this study there may be differences in these populations for reasons that could not be anticipated at the outset of the study. Because of the international reputation of prostate cancer treatment at Johns Hopkins, the population of cases includes nearly 50% who have come from outside of Maryland, and 80% who have come from outside of Baltimore. In contrast, the controls are much more likely to come from Baltimore, and relatively few are from outside of Maryland. Thus, it is possible that cases and controls will differ with respect to income, education, knowledge about cancer, and general "health consciousness," all of which could induce differences in exposures. A series of analyses to evaluate this potential for bias and appropriate responses to it are described below in the section **Plans for Concluding the Study**.

Additional research spun off the parent grant. Two ongoing projects have been based on the existing case-control study and are described below.

1. Johns Hopkins Prostate Cancer SPORE (grant #2 P50 CA58236-10): "Project 5: DNA polymorphisms in genes affecting levels of oxidative stress in prostate cells: population studies of association with prostate cancer risk." This study was funded by the National Cancer Institute in September 2003 as one of five research projects in the Johns Hopkins Prostate Cancer SPORE grant. The study will use the current case-control population. Fifty genes known to be associated with production of reactive oxygen species (ROS), detoxification of ROS, or repair of DNA damage due to ROS will be evaluated. For each of the candidate genes targeted for analysis, we will genotype approximately 8 to 12 single nucleotide polymorphisms (SNPs) per gene in the cases and controls from the parent study. These analyses are ongoing in the laboratory of the SPORE Co-Principal Investigator, Dr. William Isaacs. Once these SNP analyses are completed, the data will be used to identify prostate cancer risk-modifying genes by performing association analyses of genotype frequencies in cases and controls. We will correlate this data with the data on dietary intake, biomarkers of oxidative stress (e.g. serum malondialdehyde), oxidative damage (e.g. deoxyguanosine-malondialdehyde DNA adducts, protein oxidation), and antioxidants (e.g. lycopene, tocopherols, carotenoids) from the parent study to determine which genes modify the association of these exposures with risk. As described above, these biomarker analyses have recently begun as part of the parent grant in the lab of Dr. A. Venket Rao at University of Toronto. Finally, the associations identified in the case-control population will be validated in two independent study populations: a cohort study in Washington County, MD (Kathy Helzlsouer, PI), and an African American case-control study population collected at Howard University (Rick Kittles, PI).

2. Serum leptin and prostate cancer risk. This pilot study is a collaboration with Dr. Prakash Rao at the University of South Alabama. Obesity is a potential risk factor for prostate cancer, and is closely correlated with dietary fatty acid intake, a potential source of oxidative stress. Circulating leptin levels are correlated with obesity and androgen activity, and the prostate contains receptors for leptin. Studies *in vitro* have shown that leptin stimulates prostate cancer cell proliferation (Somasundar 2004), but epidemiologic studies have been few and inconsistent (Stattin 2001; Stattin 2003). We decided to examine leptin in a pilot prostate cancer case-control study to determine whether (a) leptin was associated with oxidative stress, and (b) whether leptin was associated with prostate cancer risk or modified associations with ROS-related exposures. A set of 35 cases was matched to 35 controls on age and race, and were sent to Dr. Rao blinded as to case-control status or any clinical or epidemiological data. Serum leptin levels were assayed using a Human Leptin direct sandwich ELISA.

Serum leptin levels were not significantly correlated with age or with the site of patient enrollment (i.e. Johns Hopkins, Georgetown, or Washington VA Hospital). Leptin was significantly correlated with BMI. Mean leptin was 7.1 for men with BMI < 25 vs. 18.4 for BMI \geq 25, $p=0.0006$. Leptin was also inversely correlated with education, being higher in men with some college or less education, mean=15.9 vs. 10.6 in college graduates or men with post-graduate education, $p=0.03$. A similar association was seen with income. However, cases and controls did not differ with respect to education, $p=0.50$. In a logistic regression model that expressed leptin as a binary variable split at the median (median=14.2), we found high leptin to be inversely associated with prostate cancer risk, OR=0.37 (95% CI: 0.13, 1.08), $p=0.07$. However, there was a significant interaction with BMI (expressed as a binary variable: < 25 vs. \geq 25), $p=0.048$ for interaction. This model gave the following results:

BMI < 25: Leptin above median vs. below median: OR=2.0 (95% CI: 0.2-25.8)

BMI \geq 25: Leptin above median vs. below median: OR=0.1 (95% CI: 0.02-0.50)

Although these results are based on small numbers and a secondary analysis of data from the original case-control study, they suggest that some of the inconsistency that has been observed in the literature concerning leptin and prostate cancer risk may reflect different distributions of body mass index.

There was no significant effect of adjustment for age, education or income. Additional analyses are planned to determine whether dietary factors may impact on these results. We also plan to expand the sample size, and evaluate the relationship between diet, leptin, and oxidative stress, once we complete the analyses of markers of oxidative stress that are being carried out by Dr. A. Venket Rao at University of Toronto. The leptin analyses were carried out by Dr. Prakash Rao at no additional cost to the study.

Plans for concluding the study. Enrollment has already exceeded the recruitment goals originally defined in the grant application (240 cases and 240 controls). We are beginning to request paraffin-embedded tumor material for analyses of deoxyguanosine malondialdehyde. We have sent dietary data for all subjects enrolled at JHU to Block Dietary Data Systems, Inc., the company that produces the dietary questionnaire and analyzes completed questionnaires for nutrient content. The nutrient data have been quantified and sent back to us for analysis. We have begun shipping serum and DNA samples for biomarker analysis to our collaborator at University of Toronto, Department of Nutrition, Dr. A. V. Rao (not the same Dr. Rao as the one who provided the pilot analysis of the samples for leptin). Serum is being analyzed for malondialdehyde, fatty acids, and antioxidant profile; lymphocytes and paraffin-embedded tissue will be analyzed for deoxyguanosine malondialdehyde DNA adducts. We expect the biomarker assays to be completed in 3-5 months. As biomarker assays are completed the data will be sent back to us at Johns Hopkins, cleaned, coded, and entered into the study database to facilitate beginning the biostatistical analysis as soon as possible.

During the conduct of the biomarker analyses we will be performing final quality control and data cleaning of the epidemiology and dietary data (e.g. identification of inappropriate responses to questionnaire items can be resolved by calling the study participant if the hard copy data do not permit resolution). We will also begin preliminary analyses to evaluate associations with case-control status and dietary data, family history of cancer, occupation, body mass index, tobacco, alcohol, tea drinking, supplement use, and medication use. These analyses will also carefully evaluate differences in case-control status with respect to income, education, and other medical conditions to determine whether the different population base of cases and controls is likely to induce bias. In particular, we will evaluate whether different distributions with respect to income and education are present, and if so, do they induce overall differences between cases and controls with respect to factors related to health knowledge and health consciousness. These analyses will help us determine factors that may need to be adjusted in the biomarker and dietary data analyses. We will also determine whether any observed case-control differences in these factors are maintained when we look at case and control subgroups from the same source populations. We will also conduct analyses separately within the case and control groups to determine whether exposures of interest (e.g. dietary data, tobacco, alcohol, supplement use) differ between source populations. These analyses will all help us determine the likely presence and direction of selection biases, and plan our approach to estimate the likely impact and possible corrective strategies. Stratified analyses will be undertaken within the subgroup of patients that represents the Maryland catchment area for Johns Hopkins. The above analyses will be completed by the time all biomarker data have been received. From that point, we expect biostatistical analyses correlating biomarker data with dietary data and case-control status to take 2 months, at which time we will submit an Amended Final Report to USAMRAA.

CONCLUSION

This study has surmounted a number of setbacks and has exceeded its accrual goals and formed the basis for additional peer-reviewed funded and unfunded research. Although there will be a delay in completing planned analyses and preparing reports and publications, the study has assembled a rich resource of data and biological specimens, which will be used to evaluate (a) the role of oxidative stress and oxidative damage in prostate cancer, (b) the influence of dietary factors and vitamin supplements on levels of oxidative stress and oxidative damage, (c) the validity of deoxyguanosine malondialdehyde measured in lymphocyte DNA to serve as a dosimeter of levels in the prostate. This resource will be used for additional biomarker studies, both in the ongoing Prostate Cancer SPORE study, and future studies to evaluate markers of inflammation and their relation to diet, genotype, and oxidative damage. In addition, we have begun collecting clinical-follow-up data for the prostate cancer patients in the study and will continue to do so, allowing us to analyze in the future the influence of oxidative damage on prostate cancer prognosis.

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APPENDICES

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LIST OF ABBREVIATIONS AND ACRONYMS

ELISA	Enzyme-linked immunoabsorbent assay
GU	Georgetown University Medical Center
IRB	Institutional Review Board
MDA	malondialdehyde
dG-MDA	deoxyguanosine malondialdehyde
NCI	National Cancer Institute
PIN	Prostatic intraepithelial neoplasia
PSA	Prostate-specific antigen
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
SPORE	Specialized Program of Research Excellence
VA	Veterans Administration Hospital
WHC	Washington Hospital Center
WIRB	Western Institutional Review Board

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